PTO 08-2772

CC=CN
DATE= 20020703
KIND= Patent
PN= 1356552

BIOCHEMICAL PROTEIN CHIP AND PREPARATION

[Shengwu Huaxue Xing Danbaizhi Xinpian Jiqi Zhizuo Fangfa]

GUO Zhanjun, ZHAO Hua, GUO Aiqin, YANG Huanyun

UNITED STATES PATENT AND TRADEMARK OFFICE

Washington, D. C. March 2008

Translated by: Schreiber Translations, Inc.

Country : People's Republic of China

Document No. : 135652

Document Type : Patent

Language : Chinese

Inventor : GUO Zhanjun, ZHAO Hua, GUO Aiqin, YANG Huanyun

Applicant : GUO Zhanjun

IPC : G01N 33/53

Application Date : 20011108

Publication Date : 20020703

Foreign Language Title : Shengwu Huaxue Xing Danbaizhi Xinpian Jiqi Zhizuo Fangfa

English Title : Biochemical Protein Chip and Preparation

Claims

What is claimed is:

1. A biochemical protein chip mainly consisting of vector substrate containing active genes and sampler cured on the vector substrate, wherein the sampler on the vector substrate also contains well mixed and closely contacted target probes and stabilizer, wherein the above sampler is in array shape with the number of arrays of 10~10000/cm^2 and the size of array of 5~500μm.

2. The biochemical protein chip in Claim 1, wherein the stabilizer in the said sampler consists of mycose, alkyl polyalcohol, and antiseptic agent, wherein the weight ratio of target probe and mycose in the above stabilized sampler is 1:10~30, the weight ratio of target probe and antiseptic agent is 1:0.01~1, and the content of alkyl polyalcohol in the above stabilized sampler solution is 10~50% (V/V).

3. The biochemical protein chip in Claim 1 or 2, wherein the components of the stabilizer include 30~50g/L mycose, 30~50%(V/V)

[1] Numbers in the margin indicate pagination in the foreign text.

alkyl polyalcohol, 0.1~5g/L antiseptic agent, water solvent and other extremely small amount of impurities.

4. The biochemical protein chip in Claim 1, 2, or 3, wherein the said alkyl polyalcohol in the stabilizer is C_2~C_{10} alkyl polyalcohol and the said antiseptic agent is sodium azide, thiomersalatum, chlorhexidini, or benzalkonium bromide.

5. The biochemical protein chip in Claim 1, wherein the target probe in the above sampler is antigen, antibody (including monoclonal antibody, anti-human IgM capture antibody, anti-human IgG antibody or second antibody), drug receptor, polysaccharide lectin, cell, or tissue.

6. The biochemical protein chip in Claim 1, wherein the target probe in the above sampler is egg yolk antibody IgY.

7. A method for preparing the biochemical protein chip described in Claim 1, wherein different types of synthesized or purified target probes and the above stabilizer are blended in proportion to become the sampler, wherein the above sampler is printed or sampled on the corresponding situs of the vector substrate while bovine calf serum is used to close the active genes having no bonding samplers on the vector substrate.

8. The method in Claim 7, wherein 1~10mg dry weight target probe,

30~100mg mycose, 0.01~1.0mg antiseptic agent, and 100~500μl alkyl polyalcohol are mixed, added with distilled water to 1ml, and dissolved to become stabilized sampler, or 30~100mg mycose, 0.01~1.0mg antiseptic agent, and 100~500μl alkyl polyalcohol are added to 500~900μl solution containing 1~10mg/ml target probe.

/2

9. The method in Claim 7 or 8, wherein the weight ratio of target probe and mycose in the said stabilized sampler is 1:10~30; the weight ratio of target probe and antiseptic agent is 1:0.01~1, and the content of alkyl polyalcohol in the above stabilized sampler solution is 10~50% (V/V).

10. The biochemical protein chip in Claim 1, wherein the chip is applicable for parallel detection, discrimination, verification, and protein studies to antigens or antibodies, drugs or receptors, polysaccharides or lectins, tissues or cells.

Descriptions

Biochemical Protein Chip and Preparation

The invention falls in life science field and relates to a device for parallel detection, discrimination, and verification to proteins, antigens or antibodies, drugs or receptors, polysaccharides or lectins, tissues or cells. The invention especially relates to a biochemical protein chip as well as a method for preparing the biochemical protein chip.

The existing biochip refers to microarrays of high density DNA, antigens, antibodies, cells, or tissues wrapped on solid phase vectors. It takes advantage of the specific mutual identification capability of biomacromolecules to orderly arrange the molecules on solid phase vector substrate (membrane, lass slide, silicon chip etc.) to react or hybrid simultaneously with samples in detection and marked biomolecules in order to obtain substantial and useful biological information through automatic reading device.

The DNA chip in biochip is also called gene chip, which is a high density oligonucleotide (DNA probe) microarray. It applies in situ combination synthetic chemistry and microelectronic chip

lithography technique, or makes use of other methods to orderly cure substantial amount of specific-sequence DNA segments (probes) on glass or silicon chip, thus constituting a DNA chip stored with extensive life information.

Peptide chip in biochip is made by fixing peptide or protein on specific solid phase vector substrate. It takes advantage of the theorem of specific combination of peptide or protein with ligand molecules (such as antigens or antibodies) for specificity reaction with serum or sample solution, and uses respective marking molecules for large scale detection of antigens, antibodies or proteins and polypeptide segments.

Peptide chip is also called protein chip. Following the same theorem of gene chip, the peptide chip combines immunological marking reaction and applies different methods to directly sample a variety of proteins (antigens or antibodies) of different sources on a solid phase vector substrate so that the proteins are firmly bonded with the vector substrate to form a protein microarray, i.e., the protein chip. The antigen or antibody substances are mostly proteins while the combination of antigens and antibodies are specific combinations. Therefore, various marks can be applied on the antigens or antibodies to detect respective antibodies or antigens. The antigens or

antibodies conduct specific immunological reaction with specially marked protein molecules (antibodies or antigens) on the protein chip, thus achieving mutual test of antigens or antibodies through detection to markers. Characterized by high volume, miniaturized, parallelized, and automatic detection, this technique is broadly applicable to detection of clinical diseases, and research on drug screening and protein interactions.

/2

Ciphergen Biosystems Inc. was one of the companies that developed protein chips in early stage in the world. It presented that protein chips can be divided into two types--chemical protein chips and biochemical protein chips (Merchant M et al., Electrophoresis, 2000, 21: 1164~1177). The concept of chemical protein chip is developed on the basis of classic chromatography materials (reversed chromatography, ion exchange chromatography, metal chelate chromatography etc.). The fundamental principle of chemical protein chip is that the protein chip laid with related medium can bond with proteins in the sample through hydrophobic force, electrostatic force, and covalent bond of the medium, and remove impure proteins through special eluent while retaining the concerned proteins for analysis. This type of chemical protein chip has poor

specificity. Biochemical protein chip bonds bioactive molecules (antigen or antibody, receptor, ligand, etc.) on the chip surface for capturing target protein in the sample. Since biochemical protein chip has high specificity and high bioactive molecular diversity, its application scope and application prospect are obviously better than chemical protein chip. At present, the protein chips produced and promoted by Ciphergen Biosystems Inc. are still mostly limited to chemical chips. Arrenkov et al. (Arenkov P et al., Anal Biochem, 2000, 289: 123~131) fix probe proteins on micropolyacrylamide gel to make protein chip, and take advantage of electrophoresis to capture target proteins bonded specifically with probe proteins in the sample.

Protein is more difficult to synthesize than DNA, and even more difficult to synthesize on the surface of solid phase supporter. In addition, the proteins positioned on solid surface may easily lose activity due to the change of spatial conformation, making it hard to keep the bioactivity of proteins on solid surface and making the protein chip much more difficult in both manufacturing and application than DNA chip. Therefore, how to keep the bioactivity of proteins in protein chip manufacturing process has become a bottleneck technique constraining the development of protein chips.

Recently, Macbearth et al. (Macbearth G et al., Science, 2000,

289: 1760~1763) made breakthrough in the manufacturing of protein chips. They first coated a layer of bovine calf serum albumin on the surface of glass slide (bovine calf serum albumin provides hydrophilic surface to prevent denaturation of probe proteins on the surface), and then use robot to sample probe proteins on the glass slide surface, forming about 150~200μm diameter high density microarray (1600 points/cm^2). After that, the probe proteins are anchored on BSA membrane surface through chemical reaction. This preparation process has not only employed robot for DNA chip manufacturing and ordinary commercial glass slide, but also successfully solved the problem of the loss of activity of proteins on solid surface.

/3

Three issues need to be addressed on protein chips. First, the chip has to keep the protein activity; Secondly, the chip has to ensure correct positioning of protein; and thirdly, the chip has to be compatible with the existing mRNA microchip research tools. In this regard, the solution of Gavin MacBearth and Stuart Schreiber is to first add 40% glycerin to sampled sodium-upgrade solution to prevent protein denaturation, and then coat a layer of reagent reacting with azyl on the membrane surface so that the protein azyl terminal or residue of lysine can react with the reagent and thus be fixed on

the membrane. Each type of protein can be positioned in a variety of different directions to ensure correct positioning. After that, the protein is closed with BSA to lower the background on the membrane and then hybridized with the proteins in test. Gavin MacBearth and Stuart Schreiber apply the above method to have prepared 1600 points/cm^2 protein microarray for studying protein interactions and action with small molecules (MacBearth, G. Schreiber, S.L., Printing proteins as microarrays for high-throughput function determination. Science, 2000, 289: 1760~1763 PubMed).

Although the above biochemical protein chip preparation method is simple and practical, the 40% glycerin added to the protein probe solution leads to the increase of solution viscosity, making it very difficult to truly achieve sodium-upgraded quantitative sampling. Besides, glycerin solution does not provide comprehensive protection to protein activity. Glycerin is merely protective to protein activity in solution state and is ineffective in dry state. Moreover, the samples containing glycerin are hard to dry up. The key link in protein chip preparation process is to keep protein activity, especially in dry state, which is hard to achieve with merely 40% glycerin. During the drying process and in the dry sate of protein probe microarray, it is very easy to cause irreversible denaturation or loss of activity

of protein probes, thus affecting the detection sensitivity and specificity of the protein chip and even affecting the stability and storage period of the protein chip.

The objective of the invention is to provide a high sensitivity, high accuracy, stable-to-store, simple-to-make, suitable for mass production biochemical protein chips.

The invention also relates to a method for manufacturing the above biochemical protein chips.

The invention is realized through the following processes:

The invention provides a biochemical protein chip for detecting the existence of antigens or antibodies, drugs or receptors, polysaccharides or lectins, tissues or cells in the samples in test. The chip mainly consists of a vector substrate and sampler cured on the vector substrate. The sampler on the vector substrate also contains well blended and closely contacted target probes and stabilizer simultaneously. The above sampler is in array shape with the number of arrays of 10~10000/cm^2 and the size of array of 5~500μm. The target probes in the above sampler are used for capturing target substance in the sampler, while the stabilizer is used to stabilize the target probe in the sampler. The target probes are antigens or antibodies, drugs or receptors, polysaccharides or lectins, tissues

or cells. The stabilizer consists of mycose, alkyl polyalcohol and antiseptic agent.

/4

In the invention, the sampler also simultaneously contains egg yolk antibody IgY target probe and stabilizer.

The preparation method is as follows: Blend different types of synthesized or purified target probes and the above stabilizer in proportion to become sampler. Print or sample the above sampler to corresponding situs on the vector substrate, and then use bovine calf serum to close active genes having no bonding samplers on the vector substrate.

One characteristic of the preparation method of the invention is that the above synthesized or purified target probes are undergone stabilization treatment with the above stabilizer. The preparation procedure is as follows: Take 1~10mg dry weight target probe, 30~100mg mycose, 0.01~1.0mg antiseptic agent, and 100~500μl alkyl polyalcohol. Add distilled water to 1ml and dissolve to become stabilized sampler, or add 30~100mg mycose, 0.01~1.0mg antiseptic agent, and 100~500μl alkyl polyalcohol to 500~900μl solution containing 1~10mg/ml target probe. The weight ratio of target probe and mycose in the said stabilized sampler is 1:10~30; the weight ratio of target probe and

antiseptic agent is 1:0.01~1, and the content of alkyl polyalcohol in the above stabilized sampler solution is 10~50% (V/V).

The above procedure can be applied to prepare stabilized sampler for different types of target probes. The alkyl polyalcohol and antiseptic agent can stabilize bioactivity of target probes in liquid state, while the mycose can stabilize the bioactivity of target probes in the dry-up process or even in dry state. The three substances perform functions jointly to avoid irreversible denaturation or loss of activity of target probes in either liquid state or dry state, thus enhancing the detection sensitivity, specificity, stability, and storage period of the invention and achieving the objective of specific, traced, and parallel detection of a variety of antigens, antibodies, and diseases.

/5

In the invention, the vector substrate contains stabilized target probe sampler. Once the serum or sample in test is added, the target probe will conduct specific immunological reaction with the antibody or antigen in the serum or sample to be tested and, upon washing out unreacted substances, further react with the added marker. By removing the redundant marker, the reaction result can be observed through chromogenic reaction or luminous reaction. If the serum or

sample in test contains respective antibody or antigen, the antibody or antigen will be captured by the target probe contained on the vector substrate. The antibody or antigen then combines specifically with the marker to occur chromogenic reaction or luminous reaction with positive result. If the serum or sample in test does not have the respective antibody or antigen, the serum or sample will not be captured by the target probe contained on the vector substrate and thus cannot be specifically bonded by the marker. The marker is then washed away without occurring chromogenic reaction or luminous reaction. The test result will be negative. In the invention, the stabilizer is employed for stabilization treatment to target probe, leading to simple and fast operation, accurate and reliable result, high sensitivity and specificity, and high stability in storage. The detection method offers comparable room temperature stability with the dot filtration assay method, and is applicable to parallel detection, identification and verification of antigens or antibodies, drugs or receptors, polysaccharides or lectins, tissues or cells using immunolabelling methods such as sandwich detection method, dual-situs one-step method, indirect method and capture method. The biochemical chip is characterized by simple structure, easy operation, high stability, sensitivity, accuracy, and specificity. Besides, the

preparation process is simple and suitable for large-scale automated production.

The invention is further described in detail as follows:

The invention provides a biochemical protein chip mainly consisting of vector substrate and sampler cured on the vector substrate, wherein the sampler on the vector substrate also contains well mixed and closely contacted target probes and stabilizer, wherein the above sampler is in array shape with the number of arrays of 10~10000/cm^2 and the size of array of 5~500μm. The target probe contained in the above sampler is used to capture the substance to be detected in the sample in test. The stabilizer is the stabilizer of the above target probe and is used to stabilize the target probe in the sampler. The target probe in the above sampler is antigen, antibody (including monoclonal antibody, anti-human IgM capture antibody, anti-human IgG antibody or second antibody), drug receptor, polysaccharide lectin, cell, or tissue. The said stabilizer consists of mycose, alkyl polyalcohol, and antiseptic agent. The component contents are: mycose 30~50g/L, alkyl polyalcohol 30~50%(V/V), antiseptic agent 0.1~5g/L, water solvent, and other extremely small amount of impurities. the said alkyl polyalcohol in the stabilizer is C_2~C_{10} alkyl polyalcohol and the said antiseptic agent is sodium

azide, thiomersalatum, chlorhexidini, or benzalkonium bromide.

Another characteristic of the invention is that the sampler also contains simultaneously egg yolk antibody IgY target probe and stabilizer. Egg yolk antibody IgY is stable in nature, resistant to acid and heat. It does not activate endogenous complement, nor combine with complement and rheumatoid factor. Therefore, it will not induce endogenous interference, thus meeting the requirements for stability, sensitivity, and accuracy. Well purified specific egg yolk antibody IgY can be stored at room temperature for 6 months without change of antibody activity. In the presence of the stabilizer of the invention, the bioactivity of the yolk antibody IgY in the sampler of the invention remains stable for 1~2 years without any change.

/6

The method for preparing the biochemical protein chip of the invention is as follows: Blend different types of synthesized or purified target probes and the above stabilizer in proportion to become the sampler. Print or sample the above sampler on the corresponding situs of the vector substrate. Use bovine calf serum to close the active genes having no bonding samplers on the vector substrate.

The above vector substrate is designed to use different

activating reagents to bond a variety of active genes on the surface of the vector substrate through chemical reaction to form covalent bond with sampler, thus forming affinity vector substrate having different biological specificities for fixing and detecting different active biomolecules such as proteins, polypeptides, enzymes, antigens, antibodies, drugs and receptors, polysaccharides or lectins, tissues or cells. For different vector substrates, different activation reagents can be applied to activate the surface of vector substrate. For example, for vector substrate with carboxyl surface, 1-ethyl-3-(3-dimethylamine) Carbodiimide Hydrochloride (EDC) and N-hydroxy Sulfosuccinimide (NHS) can be used to change the surface into active ester before coupling the surface with different biomolecules in the sampler. By taking similar method known in the field, the surface of vector substrate containing hydroxy, azyl, aldehy, diazanyl, and sulfhydryl groups can be activated into active groups and undergone different bonding reactions with the sampler, thus fixing the bioactive molecules in the sampler on the vector substrate in the form of covalence combination.

One characteristic of the preparation method of the invention is that the above synthesized or purified target probes are undergone stabilization treatment with the above stabilizer. The preparation

procedure is as follows: Take 1~10mg dry weight target probe, 30~100mg mycose, 0.01~1.0mg antiseptic agent, and 100~500μl alkyl polyalcohol. Add distilled water to 1ml and dissolve to become stabilized sampler, or add 30~100mg mycose, 0.01~1.0mg antiseptic agent, and 100~500μl alkyl polyalcohol to 500~900μl solution containing 1~10mg/ml target probe. The weight ratio of target probe and mycose in the said stabilized sampler is 1:10~30; the weight ratio of target probe and antiseptic agent is 1:0.01~1, and the content of alkyl polyalcohol in the above stabilized sampler solution is 10~50% (V/V). The said target probe in the above sampler is antigen, antibody (including monoclonal antibody, anti-human IgM capture antibody, anti-human IgG antibody or second antibody), drug receptor, polysaccharide lectin, cell, or tissue. The target probe in the above sampler is egg yolk antibody IgY. The above stabilizer consists of mycose, alkyl polyalcohol, and antiseptic agent. The components of the stabilizer include 30~50g/L mycose, 30~50%(V/V) alkyl polyalcohol, 0.1~5g/L antiseptic agent, water solvent and other extremely small amount of impurities. The said alkyl polyalcohol in the stabilizer is C_2~C_{10} alkyl polyalcohol and the said antiseptic agent is sodium azide, thiomersalatum, chlorhexidini, or benzalkonium bromide.

/7

The stabilizer mechanism in stabilizing the bioactive molecules in the sampler is as follows: mycose is a non-reductive disaccharide with extremely stable chemical property. It is not toxic and not harmful, and will not become carameled. It is capable of protecting biological cells and bioactive substances from damage of activity in adverse environment such as dehydrated, draught, high temperature, frozen, high osmotic pressure, and toxic reagent conditions. The high efficacy bio-protective effect of the stabilizer in resisting dehydration has something to do with the formation of glass state of mycose. The bioactive molecules may take advantage of the trend of glass transition of mycose to form amorphous continuous phase, which is similar to that of glass state ice in structure. In this structure, the motion or denaturation reaction of bioactive molecules is very weak, thus making the activity of biomolecules stabilized. Besides, the hydroxy of mycose and alkyl polyalcohol combines with biomolecules in the form of hydrogen bond, which has enhanced the thermodenaturation temperature of biomolecules and improved thermal stability of biomolecules. Furthermore, mycose and alkyl polyalcohol molecules can wrap around biomolecules or fill in the spatial structure of biomolecules, especially in the nearby area of active part of biomolecules to form glass state, fix the spatial structure

of biomolecules, and thus avoid deactivation of biomolecules. On the other hand, it is usually regarded that proteins, saccharide, fat substances and other biomacromolecules are all contained by a layer of water membrane, which is absolutely necessary for keeping the structure and function of biomolecules. When biomolecules lose the water membrane that keeps the structure and function of the molecules, the mycose and alkyl polyalcohol can link the anhydrous part of the biomolecules in the form of hydrogen bond to form a layer of protective membrane to replace the lost structural water membrane, thus stabilizing the spatial structure and bioactivity of biomolecules. The antiseptic agent, especially sodium azide, can inhibit the growth of bacteria in the sampler. The above mycose, alkyl polyalcohol, and antiseptic agent jointly form the stabilizer of the target probe. The alkyl polyalcohol and antiseptic agent can stabilize bioactivity of target probes in liquid state, while the mycose can stabilize the bioactivity of target probes in the dry-up process or even in dry state. The three substances perform functions jointly to avoid irreversible denaturation or loss of activity of target probes in either liquid state or dry state, thus enhancing the detection sensitivity, specificity, stability, and storage period of the invention and achieving the objective of specific, traced, and

parallel detection of a variety of antigens, antibodies, and diseases.

/8

In the invention, the vector substrate contains stabilized target probe sampler. Once the serum or sample in test is added, the target probe will conduct specific immunological reaction with the antibody or antigen in the serum or sample to be tested and, upon washing out unreacted substances, further react with the added marker. By removing the redundant marker, the reaction result can be observed through chromogenic reaction or luminous reaction. If the serum or sample in test contains respective antibody or antigen, the antibody or antigen will be captured by the target probe contained on the vector substrate. The antibody or antigen then combines specifically with the marker to occur chromogenic reaction or luminous reaction with positive result. If the serum or sample in test does not have the respective antibody or antigen, the serum or sample will not be captured by the target probe contained on the vector substrate and thus cannot be specifically bonded by the marker. The marker is then washed away without occurring chromogenic reaction or luminous reaction. The test result will be negative. In the invention, the stabilizer is employed for stabilization treatment to target probe, leading to simple and fast operation, accurate and reliable result,

high sensitivity and specificity, and high stability in storage. The detection method offers comparable room temperature stability with the dot filtration assay method, and is applicable to parallel detection, identification and verification of antigens or antibodies, drugs or receptors, polysaccharides or lectins, tissues or cells using immunolabelling methods such as sandwich detection method, dual-situs one-step method, indirect method and capture method. The biochemical chip is characterized by simple structure, easy operation, high stability, sensitivity, accuracy, and specificity. Besides, the preparation process is simple and suitable for large-scale automated production.